

Translation

Introduction

Protein synthesis is the elongation of amino acid chain. Each amino acid is added one at a time. The growing chain is shifted within the ribosomes from the previous tRNA to the new one. These reactions form peptide bonds—strong bonds—which are energy-rich. To do this, tRNAs need priming with the correct amino acid and energy for the bond, a step called **activation**. Subsequently, these tRNAs can be used in protein synthesis via a three-step process: **initiation** (binding of ribosomes to the mRNA), **elongation** (reading the mRNA and adding to the growing amino strand), and **termination** (receiving the stop signal, dissociation of all molecules, and release of the now-finished amino sequence). After translation is through, post-translational changes occur to bring the protein to its final form.

Activation of tRNA

Activation is the process by which a **high-energy bond** is made between the **amino acid** and the **3' end of tRNA**. An activated tRNA is required by the ribosome (an unactivated tRNA wouldn't have an amino acid to add to the chain). Activation not only installs an amino acid to the tRNA that corresponds to the anticodon, but it also instills the energy needed to make the peptide bond of the growing chain.

Activation takes an amino acid, free floating in cytoplasm, and sticks it onto the right tRNA. The enzyme **aminoacyl-tRNA-synthetase** ensures this specificity. For every amino acid there is a different synthetase, so the step of activation is very specific.

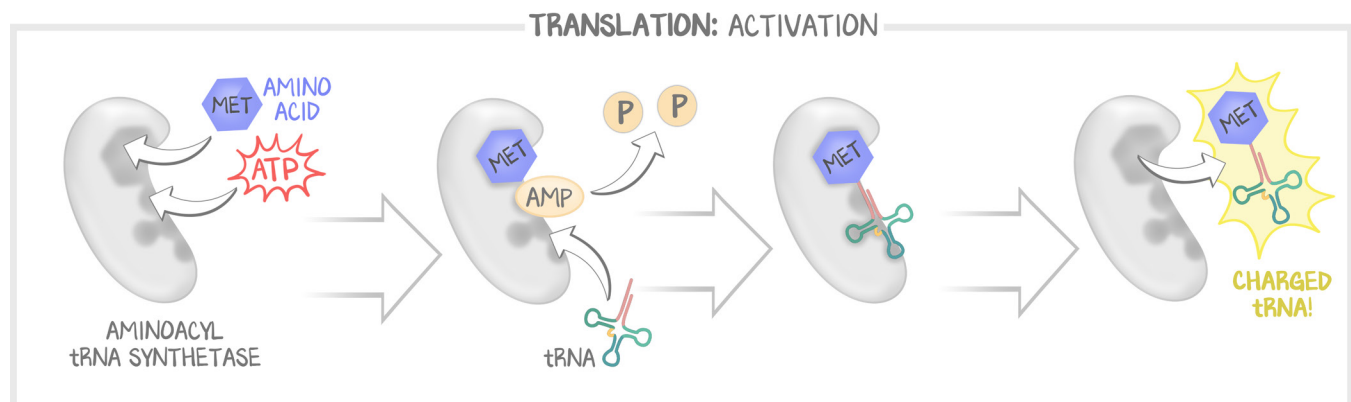


Figure 13.1: Activation

This is a two-step process. The first step is to activate the amino acid with AMP, and the second step is to use that energy from the AMP to store a high-energy bond between the tRNA and the amino acid. That energy will eventually be used to catalyze the elongation of the amino acid chain.

An amino acid has the amino (N-terminus) to the left, and the carboxyl (C-terminus) to the right. The synthetase grabs hold of the amino acid and ATP, connecting the amino acid to AMP (discarding two phosphates). The synthetase holds onto that high-energy amino acid and lets a tRNA into the binding site. Using the energy from the phosphate bond to AMP, the synthetase transfers the amino acid to the tRNA. While AMP is released, the energy is still stored in the tRNA-amino bond, and that tRNA is said to be "activated." At this point, there is a free amino terminus dangling off the end of the tRNA-amino acid combo, and the carboxy terminus is attached to the tRNA, AND that C-terminus-tRNA bond has enough energy to make a peptide bond later. This orientation becomes relevant during elongation.

Initiation of Translation

Two ribosomal subunits come together on the mRNA. In eukaryotes, that is the 40s and 60s subunits forming an 80s (eukaryotes are even) subunit. In prokaryotes the 50s and 30s ribosomal units join to make a 70s (prokaryotes are odd).

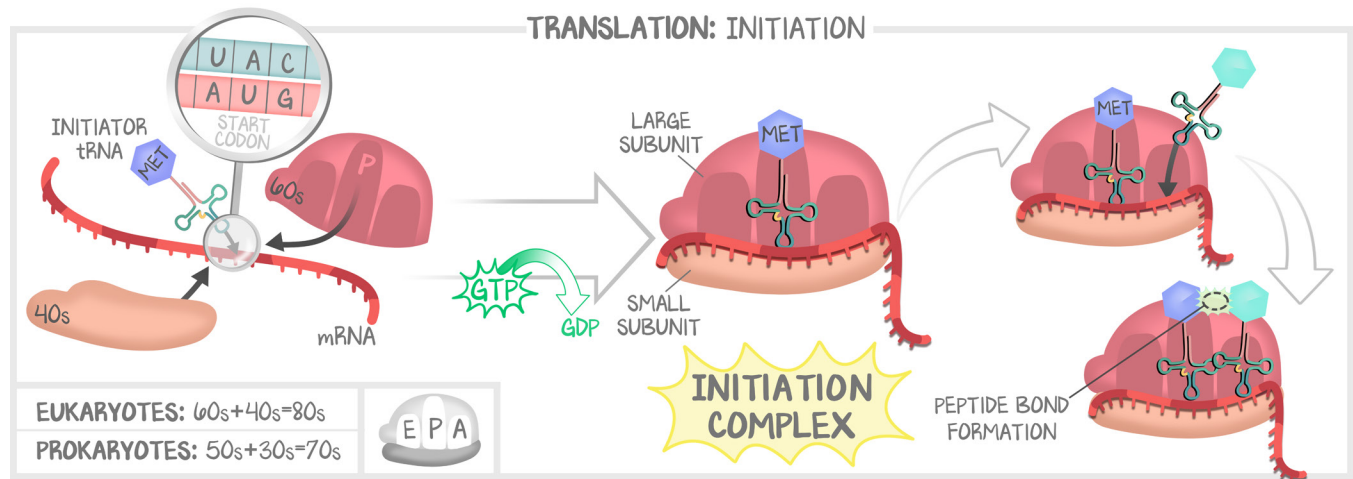


Figure 13.2: Initiation

Progression through initiation and associated sites and players.

The signal for subunits to form, the way they know where to connect and start reading, is either the **5'-methyl-guanine cap** (eukaryotes) or a **Shine-Dalgarno sequence** (prokaryotes). Eukaryotes have only one consecutive sequence to translate while prokaryotes may have a polycistron with multiple starts and stops for ribosomes. Initiation factors bind to the initiator tRNA complex, and use **GTP to get started**. The details of these factors and complex aren't relevant.

The next signal to the ribosome is "start building." That start signal is UAG; it codes for methionine. The **methionine** is formylated in prokaryotes, but not in eukaryotes. When UAG is encountered, initiation of peptide synthesis is done by initiation factors (called eIF for eukaryotic initiation factor).

Sites

The ribosome can be seen as having three sites for tRNA interaction. These are the **aminoacyl site**, the **peptide site**, and the **exit site**. The exit site isn't a site at all, it's just the ejecting of the tRNA that was in the P site out of the ribosome as the ribosome translocates. That's the last time we'll mention the E site.

The **A site** is where a new, single amino acid is brought in by its tRNA. One tRNA molecule binds to one codon and is carrying one amino acid. The **P site** is where the previous tRNA is. The previous tRNA has every amino acid in the sequence attached to it, N-terminus the farthest left (towards start), the most recent amino acid carboxy terminus attached to the tRNA.

In elongation, the amino acid sequence will be transferred from the tRNA at site P (hydrolysis of the carboxy-tRNA bond) and added to the new amino acid's amino-terminus at site A.

The only time an amino acid is added to the P site is the first amino acid, methionine. All subsequent amino acids have the entire amino acid sequence added to the new one, at the A site.

Elongation

Elongation is the process by which the already-made amino acid sequence is added to the incoming amino acid. The carboxy terminus of the amino acid strand that is currently on the 3' end of the outgoing tRNA is transferred to the new amino terminus of the incoming tRNA. The new tRNA must be accepted, the transfer made, and the old tRNA ejected. This is made possible by the ribosome actually shifting over one codon.

A **peptide bond** is formed between the old carboxy terminus and the new amino terminus. The carboxy terminus of the so-far-made strand is attached to its tRNA at site P. The amino terminus is attached to the rest of the strand. The carboxy terminus of the incoming amino acid is attached to its tRNA, but its amino terminus is free-floating at site A. That's the target—separate the already-grown strand from its tRNA and move it to the open amino terminus on the new one. This effectively moves the entire strand over to the new tRNA from the old tRNA at P site to the new tRNA at A site. The energy for that strong peptide bond comes from the **activation step**, above.

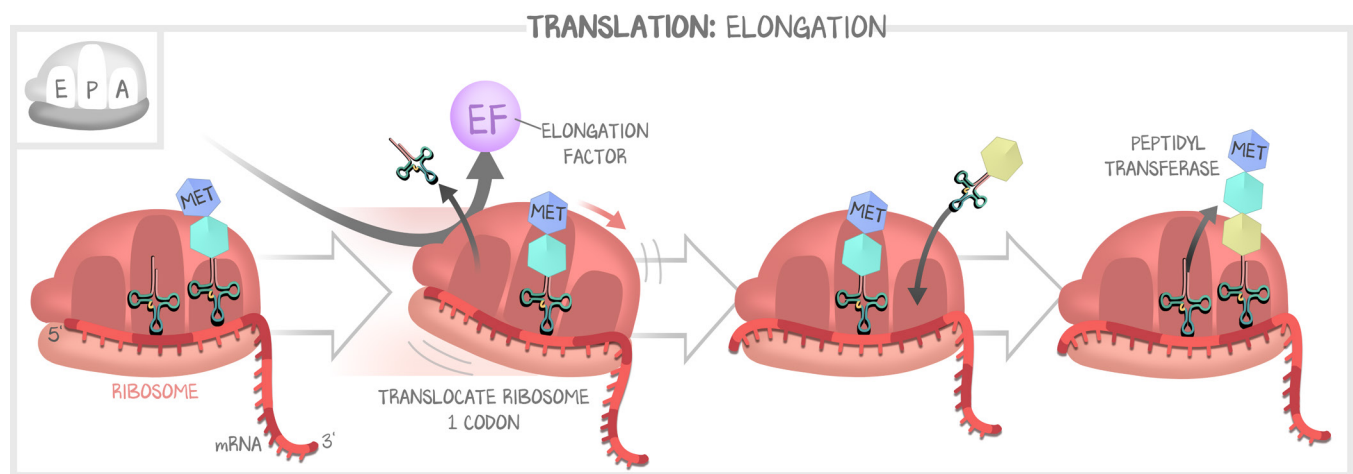


Figure 13.3: Elongation

Growing chain at the P site, one enters the A site, the peptide bond is formed, and transpeptidyl transferase moves the entire amino acid chain from the tRNA in the P to the amino acid of the tRNA at the A site. Then the ribosome moves forward three, releasing the empty tRNA, moving the tRNA with the chain to the P site, as a new charged tRNA comes into the A site.

The old tRNA attached to the amino acid sequence is at the P site. The new tRNA attached to just one amino acid is at the A site, matched to the mRNA codon by the tRNA anticodon. Time freezes. With the old tRNA at the P site, and the new tRNA at the A site, **peptidyl transferase** takes the amino acid sequence from the tRNA at the P site, and moves it over to the A site, attaching the now-free C-terminus of the growing strand to the already-free N-terminus of the new amino acid. Time unfreezes.

The ribosome **translocates** exactly 3 codons. In doing so, the old tRNA in the P site is now ejected (sometimes called the E site), and that new tRNA is moved into the P site. The A site is free, and the next tRNA binds based on its anticodon pairing to mRNA codon.

In elongation the **amino acid sequence is moved from P to A** and then **the ribosome moves over**. tRNAs are not moved—they stay still—but in doing so, appear to move relative to the ribosome. The ribosome moves the amino acid sequence from tRNA to tRNA, then the ribosome translocates itself. The only thing the tRNA does is bind its anticodon to the mRNA codon, and sit there, waiting to be discarded, recycled by activation, and start again.

Termination

Any time a **stop codon** (UAA, UAG, UGA) is encountered at the A site, peptidyl transferase hydrolyzes the carboxy terminus from its tRNA, dispatching the completed protein product out of the P site. In doing so the ribosome subunits dissociate. The mRNA is still present and likely has other ribosomal subunits still working on making more protein; the mRNA can be reused. All of the tRNAs simply need to be activated to be used again. All of the ribosomes that dissociated can reassociate and start again.

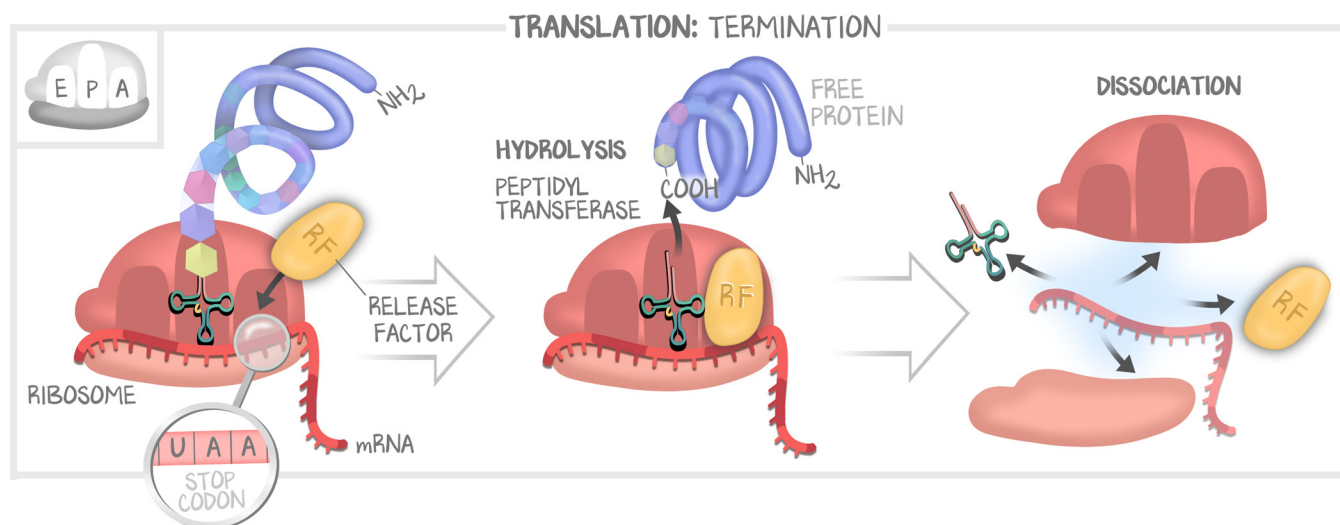


Figure 13.4: Termination

When a stop codon is encountered, the releasing factor enters the A site, hydrolysis of the amino acid chain from the tRNA in the P site occurs, and the ribosomal subunits dissociate.

Factors

Not surprisingly, there's some regulation of protein action. Like DNA polymerase and RNA polymerase, "ribosome polymerase" (aka initiation, elongation, and termination) requires that other cofactors be present, and can act to inhibit or stimulate. You need to know only that they exist: initiation factors (IF), elongation factors (EF), and termination factors (TF). We'll get into specifics as we talk about antibiotics in the next lesson.

Polysome

One mRNA strand can have several ribosomes working at once. They all start at the start. They all move 5' to 3' down the mRNA strand. They build from the amino acid N-terminus (5') to the carboxy terminus (3'). The longer the strand, the longer the ribosome has been working. The closer to the mRNA 3' end, the longer the ribosome has been working. **Polysome** is the word that refers to **multiple ribosomes going on one mRNA**.